July 4, 1954

Dr. W. B. Cherry
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Dear Bill:

I am greatly indebted to you for the preview on your very interesting work on Bacillus. It looks like much more promising material than I had thought when I first saw the Manninger-Tomcsik papers some years ago.

I realize that this ms. is not quite in final form, but as you asked for my critical comment, I had to bring up points most of which you probably have already had in mind. First of all, I doubt you need make any reference at all to J.L. unpubl., as the citations are all covered by Zinder & Lederberg '52. Also, p.l., this, rather than Stocker Z & L is the primary reference for transduction by phage.

Let me clear up terminology first. Transduction means the transmission of a fragment, by phage or any other vector (DNA or what have you.) Strictly, one should not talk about a transduced cell (though we have erred on this ourselves); I would leave it to you whether to continue this imprecision, or to substitute transinduced or transformed to describe the cell that has been altered when a genetic fragment was transduced to it.

Some of my comments relate to my personal judgment on form for publication (as in J. Bact.) rather than material comment. They are numbered per marginal marks in pencil.

1. Is this right— or is it anthracis cells plus mesentericus extracts?

2. see above

3. This whole paragraph could probably be omitted in favor of a reference
4. to some review (Austrian 1952, Bact. Rev.) among others. I think Groman distinguishes his case— at least he does now.

5. Confer/transduce. The latter term would presuppose your conclusions, and probably should not be used too freely in describing the experiments.

6. This is too critical to be passed over by "variable results". Can you present a table?

7. "transduced" see above. This is not entirely convincing evidence for lysogenicity (vs. admixture of phage + bacteria) unless the cultures were reisolated repeatedly from single colonies.

8. How were sterility tests made? How long kept? Since the other genetic markers (except possibly pathogenicity) are not so distinctive, this becomes critical.

9. I assume the motile variants were repurified before further tests.

10. Since this experiment was repeated with purified DNAse, it probably should be reduced to a line, and the experimental emphasis put on the latter. The conclusion is more important than the historical sequence.

11. In view of all this, had you not better say lysate rather than phage in describing the active material throughout the paper?

12. Your summary puts this better. There is no evidence the phage plays any role except perhaps to extract the DNA from the source bacteria.

13. Z&L '52. Best review on pneumococcal DNA is McCarty '46, Bact Rev.

14. What does unmasking mean? Is it distinct from any other mode of variation?

15. I am confused on the evidence that this is transduction, i.e., that a genetic factor must be present in the donor bacteria to result in "transmotilization". It would be necessary to compare related strains for such a comparison. Can the Ohio strain be motilized? Does it then become itself a competent donor for the transmotilization of the non-motile Ohio? As things stand now (see 6.) your system might be a more direct result of lysogenization itself, with some confusion from host-range modifications or the like.

16. The comparison is not quite valid. In Salmonella, about $1/10^6$ phage particles is competent, and a bacterium can effectively adsorb only about 10-100 phages. You have not measured the bacterial competence, as table 6 shows about 10^6 bacteria in each experiment. If phage has anything to do with it, the competence of about 10^2 phage/motilization suggests that, could you get effective adsorption at such low densities, you might get 1-10% of your bacteria transformed with a sufficient excess of phage. Your 10^{-2} per phage is very much higher than our 10^{-6}, but is not necessarily a fundamental distinction in mechanism. (We have a coli transduction now with an efficiency per phage of 10^{-1} or better!, but this is an exceptional case).(Can't you just spin out your phage at high speed and see whether the supernate is still active?)

17. Don't you wish you had some distinctive markers in your strains, though! Something like streptomycin-resistance should be easy enough, and would disqualify this conceivable source of error altogether. One might imagine that damaged spores would only germinate under special conditions.

18. Have you ever observed a spontaneous motile reversion? This would be ideal for the comparison suggested at 15.

19. My understanding is that the agent is present in lysates of phage+ sensitive bacteria. Can you get it from lysogenics directly?

20. If this is a euphemism for C.C. it is hardly secure.

In sum, I think this is an extremely provocative and competent bit of work. If there is any possibility of it, however, I think publication should be deferred until a few crucial points are cleared up:

A. Are any bacilli (in a competent system) lysogenized without being motilized? (I note here the two stages: the first is presumably induced by the phage, the second might conceivably also be induced, but more probably is selected, judging from comparable experiences with Salmonella. What happens when you plant a repurified, first stage isolate on motility agar? I think this stage corresponds to the "flares" that Stocker et al. noted, and not the trails.)

B. Point 15-6-18 above.

C. Perhaps some further attempt to remove the phage and leave activity. (Can I help in any way on this?) and

D. Point 17.

C and D are not so essential as A and B. I would hate to see the same kind of confusion and misunderstanding come out of this story as there was with diphtheria, and if a little patience can give a well-rounded account, why not wait a short while longer.

If you have an extra copy of your final version, could you let me have it (and requote it also, if you would)?

Yours truly,

Joshua Lederberg
Professor of Genetics